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Research Article

# COMPARISON OF ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF WILD AND CULTIVATED VARIETY OF *RAUWOLFIA SERPENTINA*

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**ABSTRACT**

**Objectives:** About 80% of world populations are still dependent on herbal plants. Rauwolfia is also one of the wonder drugs of India, which is used since ancient time. It contains a variety of compounds with antioxidant activity and other health benefits. A wild variety of rauwolfia is became endangered due to indiscriminate use. Hence, its cultivation and collection have been started to complete the demand of rauwolfia. Therefore, the study was designed to evaluate and compare the in vitro antioxidant and antidiabetic activity of the wild and cultivated plant of rauwolfia.

**Methods:** The methanolic extract of wild and cultivated plant was subjected to the DPPH and alpha-amylase inhibition activity for antioxidant and antidiabetic activity, respectively.

**Result:** The study revealed that the wild and cultivated variety of Rauwolfia serpentina does not have a significant difference in their antidiabetic and antioxidant activities.

**Conclusion:** On the basis of the in-vitro studies, it can be concluded that cultivated variety of the plant can be used as a substitute for a wild variety of R. serpentina.

**Keywords:** *Rauwolfia serpentina*, Endangered plant, Antioxidant activity, Antidiabetic activity.

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## INTRODUCTION

India has the oldest and richest diverse cultural tradition associated with the herbal medicinal plants for curing numerous ailments [1]. Almost, 80% of the world population is relying on herbal medicines for their health care benefits because plant-based treatments are safe, economic, accessible, reliable, and highly effective. Therefore, exponential growth in the overall demand for the herbal drugs and their products are reported globally. However, uncontrolled growth of the population and unplanned or excess use of plant species is making them endangered [2]. Approximately, 1/4$^{th}$ of all plant species in the world are at risk of being endangered or becoming extinct. Moreover, global warming and habitat destruction are also reasons of the disappearance of many plants. Some common plants which are become rare and endangered in the past 30 years due to habitat destruction are enlisted in Table 1.

In addition to this, there are some of the herbal plants which are used as substitute for the particular plants are Shatavari (*Asparagus racemosus*) is the substitution for Meda and Mahameda, Vidari (*Pueraria tuberose*) substitution for Jivaka and Rasabhaka, Ashvagandha (*Withania somnifera*) Kakoli and Ksirakakoli, Guduchi (*Tinospora cordifolia*), or Centaurea behen substitution for Jivaka, Orchis spp. which includes Munjataka (*Orchis latifolia Linn.*), Vamsha Rochana (*Bambusa arundinaceae*) or *Salvia haematode* substitution for Rasabhaka, *Orchis mascula* substitution for Meda, Gandhaprasarani (*Paederia foetida*), Musali (Sveta Musali) (*Asparagus adscendens*) substitution for Mahameda, Talamuli (Krishna Musali) (*Curculigo orchioides*) substitution for Kakoli, and *Chlorophytum arundinaceum* substitution for Ksirakakoli.

Other than these, *Rauwolfia serpentina* is one of the endangered plants. Although it also has a huge demand for its alkaloids, as well as the raw drug in the international market. The requirement of dried roots of rauwolfia is around 20,000 ton/year [3] across the globe. It is commonly known as serpentina root, Indian snakeroot and belongs to the family Apocynaceae. It is a perennial glabrous herb or undershrub widely distributed in moist areas in subtropical Himalayas and plains from Punjab eastwards to Assam, Khasia Mountains, and Deccan Peninsula ascending to 1200 meters [4]. It has been used in India, since ancient time and reported in various texts of the indigenous system of medicine such as Ayurveda, Siddha, and Unani [5]. Dried roots of the plants are used for medicinal purpose and are mostly about 8–15 cm long and 0.5–2 cm in thickness subcylindrical curved and rarely branched. The outer surface is grayish yellow to brown with irregular longitudinal fissures. The fracture is short with slight odor and has bitter taste [6]. *Rauwolfia serpentina* is being used for the treatment of snakebites, mental illness, and blood pressure and it is highly reputed for hypertension, to treat painful affection of bowels, diarrhea, dysentery, cholera and colic, dyspepsia, epilepsy, giddiness, insomnia, and vitiated condition of kapha and vata [7,8]. It has been included in the Appendix II of "The Convention on International Trade in Endangered Species of Wild Fauna and Flora" (CITES) and in the negative list of exports plants by the Government of India (Notification no. 24 (RE- 98)/1977-2002) [9].

It became endangered with extinction in India, due to its indiscriminate collection, limited cultivation, and huge industrial demand. Hence, cultivation of rauwolfia has been started to complete the demand. However, a cultivated variety of rauwolfia is never evaluated and

**Table 1: Endangered plants in India**

| S. No. | Region | Botanical name | Common name | Status* |
|---|---|---|---|---|
| 1 | Arunachal Pradesh | *Amentotaxus assamica* | Assam catkin yew | T |
| 2 | Gujarat | *Polygala irregularis* | Milkwort | R |
| 3 | Gujarat | *Lotus corniculatus* | Bird's foot | R |
| 4 | Gujarat | *Ceropegia odorata* | Jeemikanda | E |
| 5 | Himachal Pradesh | *Colchicum luteum* | Colchicicum | R, T |
| 6 | Jammu | *Nymphaea tetragona* | White Water-lily | E |
| 7 | Kashmir | *Nymphaea tetragona* | White Water-lily | T |
| 8 | Karnataka | *Psilotum nudum* | Fork fern | R |
| 9 | Karnataka | *Diospyros celibica* | Ebony tree | T |
| 10 | Kerala | *Actinodaphne lawsonii* | - | T |
| 11 | Kerala | *Pterospermum reticulatum* | Malayuram | R |
| 12 | Madhya Pradesh | *Belosynapsis vivipara* | - | R, E |
| 13 | Rajasthan | *Ceropegia odorata* | Jeemikanda | E |
| 14 | Tamil Nadu | *Pterospermum reticulatum* | Malayuram | T |
| 15 | Tamil Nadu | *Acacia planifrons* | Umbrella tree | R |
| 16 | Tamil Nadu | *Abutilon indicum* | Atibala | R |
| 17 | Tamil Nadu | *Chlorophytum malabaricum* | Malabar lily | T |

*(T: Threatened, R: Rare, E: Endangered)

**Table 2: DPPH assay**

| S. No. | Concentration (μg/ml) | Inhibition (%) | | |
|---|---|---|---|---|
| | | Wild | Cultivated | Ascorbic acid |
| 1 | 0.1 | 34.99 | 19.00 | 45.56 |
| 2 | 0.2 | 62.5 | 27.50 | 52 |
| 3 | 0.3 | 76.09 | 38.00 | 62 |
| 4 | 0.4 | 81.94 | 47.9 | 73.65 |
| 5 | 0.5 | 84.64 | 55.00 | 95.34 |

DPPH: 1,1-diphenyl-2-picrylhydrazyl

**Table 3: α-amylase assay**

| S. No. | Concentration (μg/ml) | Inhibition (%) | | |
|---|---|---|---|---|
| | | Wild | Cultivated | Acarbose |
| 1 | 0.1 | 16.68 | 13.70 | 8.1 |
| 2 | 0.2 | 21.79 | 18.73 | 25.54 |
| 3 | 0.3 | 24.32 | 20.11 | 50.43 |
| 4 | 0.4 | 26.30 | 25.43 | 63.23 |
| 5 | 0.5 | 58.43 | 51.80 | 87.71 |

compared for its therapeutic properties with wild variety. Hence, in the present study cultivated variety was evaluated and compared by *in vitro* antidiabetic and antioxidant activities.

## METHODS

### Sample collection

The roots of cultivated variety of the *R. serpentina* were collected from Herbal Garden and Herbarium Research Institute in ISM, Joginder Nagar, Himachal Pradesh. Whereas the wild variety was collected from the outskirts of Joginder Nagar, Himachal Pradesh.

### Authentication

Samples of a wild and cultivated variety of *R. serpentina* were authenticated from Guru Nanak Dev University, Department of Botanical and Environmental Sciences, Amritsar (Punjab).

### Preparation of methanolic extract

The coarse powder (40 g) of roots of *R. serpentina* was macerated with methanol (1 L; 95%) for 24 h (6 h frequent shaking and allowed to stand for 18 h). Then, the drug was filtered twice through Whatman filter paper no. 1. Then, methanol from the filtrate was evaporated by rotatory vacuum evaporator to get methanolic extracts of both samples.

### Experimental procedures for *in vitro* studies

#### *Antioxidant activity*

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was performed with 700 μL of sample and MeOH (control) was added to the same volume of a methanolic solution of a 100 μM DPPH. Mixtures were shaken vigorously and left to stand in the dark at room temperature for 20 min, and then, absorbance was read at 515 nm, using an ultraviolet spectrophotometer. Antioxidant activity was expressed as inhibition percentage (I%) and calculated using the following equation.

$$\text{Inhibition } (I\%) = \frac{Abs_{control} - Abs_{extract}}{Abs_{control}} \times 100$$

### Antidiabetic activity

Alpha-amylase inhibition assay was performed using dinitrosalicylic acid (DNSA) coloring agent. A total of 500 μl of *R. serpentina* plant extract and 500 μl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase solution (0.5 mg/ml) and 500 μl of 1% starch solution in 0.02 M sodium phosphate buffer were incubated for 10 min at 37°C. After incubation, 500 μl of sodium chloride was added to each tube at 5 s intervals, and then, 1 ml of DNSA color reagent was added to stop the reaction. These test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. Finally, this reaction mixture was again diluted by adding 10 ml distilled water following which absorbance was measured at 540 nm.

$$\text{Inhibition } (I\%) = \frac{Abs_{control} - Abs_{extract}}{Abs_{control}} \times 100$$

## RESULT AND DISCUSSION

### Antioxidant DPPH activity

Antioxidant activity was determined using DPPH assay at 0.1, 0.2, 0.3, 0.4, and 0.5 μg/ml concentration of the methanolic extract of the *R. serpentina* plant. Ascorbic acid has been used as a standard drug for the determination of antioxidant potential of a wild and cultivated *R. serpentina* plants.

The results showed a percentage inhibition of 19, 27.5, 38, 47.9, and 55 for cultivated and 34.99, 62.5, 76.09, 81.94, and 84.64 for wild at 0.1, 0.2, 0.3, 0.4, and 0.5 μg/ml concentrations, respectively (Table 2). A comparative DPPH % inhibition is plotted in Fig. 1 for wild, cultivated, and ascorbic acid. The $IC_{50}$ calculated for wild variety is 0.20 μg/ml and cultivated is 0.42 μg/ml, respectively. Hence, a wild variety of the plant showed good antioxidant activity compared to the cultivated *R. serpentine* and comparable to ascorbic acid.

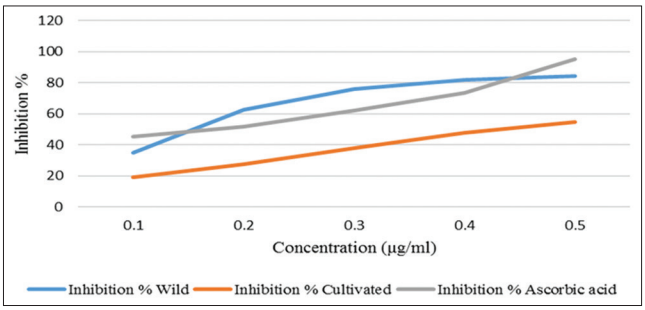


**Fig. 1: 1,1-diphenyl-2-picrylhydrazyl inhibitory % age of the wild and cultivated plant extracts**

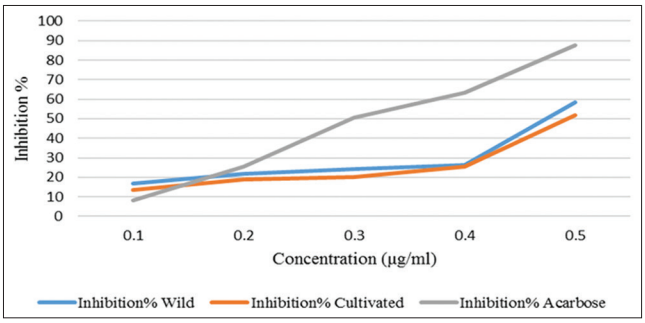


**Fig. 2: Alpha-amylase inhibitory % age of the wild and cultivated plant extracts**

**Antidiabetic activity**

There was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. The extract at a concentration 0.1, 0.2, 0.3, 0.4, and 1.0 µg/ml showed a percentage inhibition of 13.7, 18.73, 20.11, 25.43, and 51.80 for cultivated and 16.68, 21.79, 24.32, 26.30, and 58.43 for wild, respectively (Table 3). A comparative alpha-amylase % inhibition is plotted in Fig. 2 for wild, cultivated, and acarbose (a standard drug). The wild variety of the plant shows more inhibition as compared to the cultivated *R. serpentina*. The $IC_{50}$ calculated for wild variety is 0.83 µg/ml and cultivated is 0.96 µg/ml, respectively.

## CONCLUSION

Plants serve as an excellent source of various therapeutic agents. One of the major advantages of using plants is that they do not show the deleterious side effects commonly associated with other drugs. This research work explored the antioxidant as well as the antidiabetic activity of the wild and cultivated variety of *R. serpentina* plant. The study suggested that the wild source of the plant has more potential to exert antioxidant and antidiabetic activity as compared to the cultivated plant. Although the wild variety of *R. serpentina* plant is more promising, results showed that it can be substituted with cultivated variety for antioxidant and antidiabetic activity in case of need.

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